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(54) Title: DIAGNOSTICS OF DIARRHEAGENIC *ESCHERICHIA COLI* (DEC) AND *SHIGELLA* SPP.

(57) Abstract: The present invention contains a method for the identification of the diarrheagenic *E. coli* groups: ETEC (enterotoxigenic *E. coli*), A/EEC (attaching and effacing *E. coli*) EPEC (enteropathogenic *E. coli*), VTEC (verocytotoxin producing *E. coli*) and EIEC (enteroinvasive *E. coli*), and *Shigella* spp. The bacterial identification is made possible by the specific detection of the following virulence genes: *sta* and *elt* encoding heat stable enterotoxin (ST) and heat labile enterotoxin (LT) characteristic of ETEC, *eae* encoding intimin, characteristic of A/EEC, EPEC or VTEC, *bfpA* encoding bundle forming pilus (BfpA), characteristic of EPEC, *stx1* and *stx2* encoding verocytotoxin 1 and 2 (VT1 and 2) characteristic of VTEC, *ipaH* encoding invasive plasmid antigen H (IpaH) characteristic of EIEC and *Shigella* spp., and *ehxA* encoding enterohemolysin (EhxA) characteristic of some EPEC and VTEC strains. The method allows the simultaneous detection of any combination of the 8 virulence genes by one single multiplex-PCR. The method is thoroughly validated with respect to sensitivity and specificity, and showed high performance compared to other publication. The method includes an internal positive PCR control and the carry-over prevention system, UNG, which makes it ideal for routine diagnostic analyses. The method can be combined with a number of other technologies leading to even higher sensitivity and reduced time of analysis - both important parameters when diarrheagenic patient or contaminated foods are analysed.



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